LACK OF STABLE INITIATION FACTOR 3 (IF-3) BINDING TO DIMERS OF THE 30 S RIBOSOMAL SUBUNITS*

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1. Introduction

It is known that initiation factor 3 (IF-3) is endowed with ribosome dissociation factor (DF) activity [1, 2]. Recent studies have shown that IF-3 acts as anti-association factor since the equilibrium between 70 S ribosomes and 50 S and 30 S ribosomal subunits is shifted in favour of the subunits by the binding of IF-3 to free 30 S particles [3–5]. The finding that IF-3 does not *stably* bind to the 70 S monomers is consistent with this scheme [6–8]. The 30 S–IF-3 complex is dissociated during the physiological formation of the 70 S initiation complex upon addition of 50 S ribosomal subunits in the presence of fMet-tRNA, or during the formation of 70 S couples when 30 S and 50 S particles are 'forced together' at high (15 mM) Mg^{2+} concentration [6]. Therefore, an antagonism between IF-3 and 50 S particles for the binding to the 30 S ribosomal subunits seems to exist.

In the present paper we present evidence that the IF-3 binding capacity of the 30 S ribosomal particles is weakened or lost when these particles are engaged in the formation of 30 S–30 S dimers and suggest that an analogy may exist between 30 S–30 S and 30 S–50 S interactions.

2. Materials and methods

The preparation of *E. coli* (MRE 600) 30 S ribosomal subunits will be described in detail in a forthcoming paper [9]. The purification of IF-3 was carried out as previously reported [10]. Labelling *in vitro* of IF-3 was carried out by reductive alkylation [11] as previously described [7]. In a typical preparation, purified (electrophoretically homogeneous) IF-3 was concentrated to approximately 3.0 mg/ml and exhaustively dialyzed versus 0.1 M sodium borate buffer (pH 9.0) containing 0.2 M KCl and 5 mM 2-mercaptoethanol. ^{14}C-formaldehyde (57.6 mCi/mmol) was then added (0.25 mCi/1.65 mg of protein) and the mixture incubated for 30 sec in an ice-bath. After incubation sodium borohydride was added until a 2-fold molar excess of sodium borohydride over formaldehyde was reached. The solution was then dialyzed exhaustively against several changes of a buffer containing 20 mM Tris–HCl (pH 7.4); 0.5 mM dithiothreitol (DTT); 1 mM EDTA; 0.2 M NH_4Cl and 5% glycerol.

For the binding of IF-3 to ribosomes, 4 μg of [^{14}C]H_3–IF-3 (21,000 cpm/μg) were incubated with 0.30 A_{260} units of 30 S ribosomal subunits in 0.4 ml of buffer containing 10 mM Tris–HCl (pH 7.5); 100 mM NH_4Cl; 5 mM 2-mercaptoethanol, either 1 or 10 mM (cf. fig. 1) Mg $(Ac)_2$ and 0.2 mMGTP. After 10 min at 37°C, each incubation mixture was transferred onto the top of a 10–30% (w/v) sucrose density gradient made in the above buffer (either 1 or 10 mM Mg $(Ac)_2$) and centrifuged for 3 hr at 48 000 rpm at 2°C in a SW 50.1 rotor. Each gradient was pumped through the flow-cell of a Zeiss spectrophotometer and fractionated directly into counting vials. The radioactivity of each fraction

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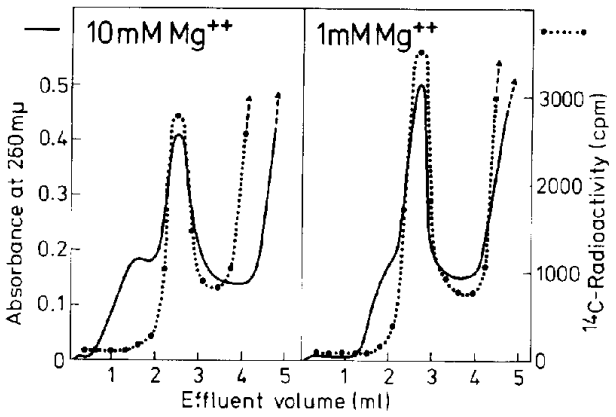

Fig. 1. Binding of [^{14}C]H_3-IF-3 to 30 S ribosomal subinits. The binding reaction and the subsequent centrifugation were carried out as described in Materials and methods (•···•···•) [^{14}C] radioactivity; (———) A_{260}. Sedimentation was from right to left.

was determined after addition of 10 ml of Bray's solution.

3. Results

The formation of dimers of the 30 S ribosomal subunits of prokaryotic ribosomes is a known, although poorly understood, phenomenon [12, 13]. In our experience, the dimerization of the 30 S ribosomal subunits of *E. coli* is partially dependent on the ribosome preparation (age, number of cycles of freezing and thawing) and on the Mg^{2+} concentration of the resuspending medium.

The binding of IF-3 to a preparation of 30 S particles which displayed a marked tendency to dimerize at high (10–20 mM) Mg^{2+} is shown in fig. 1. As seen in the figure, when the binding of radioactive IF-3 to ribosomes and the subsequent centrifugation through sucrose density gradient were carried out in a buffer containing 10 mM Mg^{2+}, a discrete A_{260} peak of 30 S-dimers sedimented faster than the 30 S peak. All the IF-3 radioactivity was associated with the 30 S peak and no IF-3 sedimented with the 30 S-dimers under these conditions. If the binding of IF-3 and the subsequent centrifugation were carried out at 1 mM Mg^{2+}, however, the A_{260} dimer peak was reduced to a shoulder, while the 30 S peak showed a corresponding increase. In addition, the increase in the A_{260} of the 30 S particles resulted in a concomitant increase in the amount of radioactive IF-3 sedimenting in the 30 S region, while the remaining fast-moving shoulder was still free of radioactivity. The possibility that the increase in radioactive IF-3 sedimenting in the 30 S region at 1 mM Mg^{2+} could merely reflect an increased binding affinity of the non-dimerizing 30 S subunits for IF-3, can be ruled out for the following reasons: a) in the experiment shown in fig. 1 a large molar excess of IF-3 over 30 S ribosomal subunits has been used so that each ribosomal subunit is 'saturated' with one molecule of IF-3; b) changes in the Mg^{2+} concentration between 1 and 15 mM do not influence the affinity of the 30 S

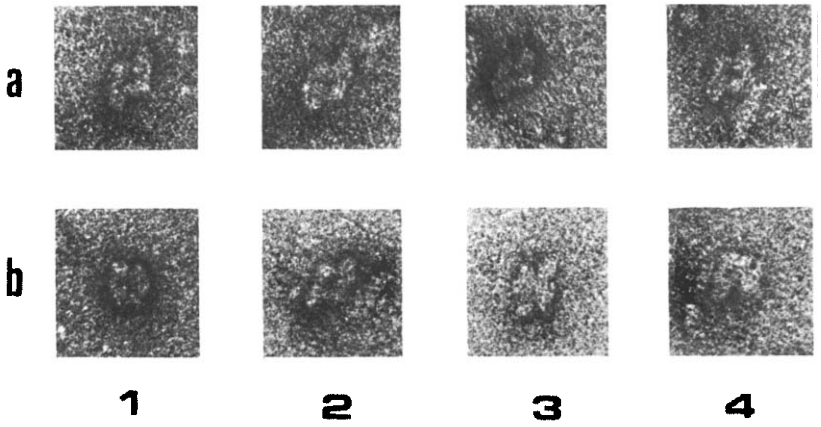

Fig. 2. Electron micrographs of negatively stained 30 S–30 S-dimers. The 30 S-dimers were dialyzed against 10 mM Tris–HCl (pH 7.6); 20 mM Mg $(Ac)_2$; 60 mM NH_4Cl and 6 mM 2-mercaptoethanol and then incubated 10 min at 37°C with 1% uranyl oxalate, pH 6.8. Magnification 400 000×. a, 1–4, b, 1–2 typical dimer formation; b, 3–4 atypical dimer formation.

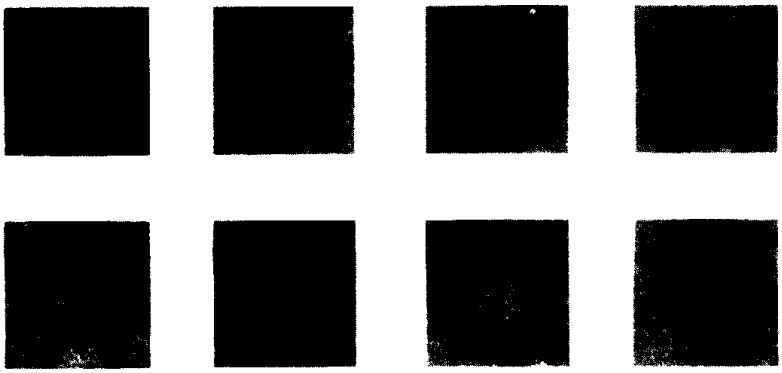

Fig. 3. Electron micrographs of negatively stained 70 S ribosomes. The 70 S monomers, in 10 mM Tris–HCl (pH 7.6); 10 mM Mg $(Ac)_2$; 60 mM NH_4Cl and 6 mM 2-mercaptoethanol, were stained with 0.5% uranyl acetate. Magnification 325 000×. The arrows indicate the 30 S subunits.

ribosomal subunits for IF-3 [6–8, 14]; c) when the particles sedimenting in the 30 S-dimer peak are isolated and dissociated at low Mg^{2+}, the 30 S ribosomal subunits so obtained are capable of binding radioactive IF-3 (not shown).

Recent studies have shown that the 30 S ribosomal subunits are ellipsoidal and sometimes curved particles of uniform size bearing a cleft in an asymmetrical position perpendicular to the long axis of the particle. This cleft, which divides the 30 S particle into two unequal lobes, is more pronounced on one side of the particle [15, 16].

Eight electron micrographs of 30 S-dimers are presented in fig. 2. Typical 30 S-dimers (over 90% of the dimers examined) appear to be particles of relatively homogeneous size and shape with the two 30 S subunits facing each other on the side where the cleft is more pronounced (fig. 2a 1–4, b 1–2). Fig. 2,b3 shows an atypical dimer in which the 30 S subunits interact 'back to back'. Furthermore, in the majority of the dimers the 30 S subunits seem to face each other in an 'antiparallel' fashion so that the large lobe of one subunit always touches the small lobe of the other (fig. 2 a 1–4, b 1–2). Fig. 2 b4 shows an exceptional case of two subunits aligned parallel to each other. In the majority of the cases, therefore, the formation of 30 S-dimers occurs through the interaction of specific regions of the 30 S particles and not through random contact between the two particles.

Examination of the electron micrographs of 70 S couples (fig. 3) seems to indicate that the 30 S subunits are in contact with the 50 S subunits through the same 'concave' surface which is responsible for the 30 S–30 S interaction (note the cleft dividing the 30 S subunits which now appears as a slightly eccentric 'hole' at the interface between the 30 S and 50 S subunit) suggesting an analogy between 30 S–30 S and 30 S–50 S interactions.

4. Discussion

The above results do not rule out the possibility of a weak interaction between IF-3 and 30 S-dimers but show that the 30 S ribosomal subunit cannot *stably* bind IF-3 if another ribosomal particle (either 30 S or 50 S) is associated with it. In almost all the 30 S-dimers observed, the 30 S particles are in contact through a specific region of their surface (most likely the same region that interacts with the 50 S subunits in the 70 S couples). It would be tempting, therefore, to speculate that also the ribosomal binding site for IF-3 [17] is localized on this part of the 30 S particles. Another explanation (which does not necessarily exclude the first one) for the lack of *stable* IF-3 binding to 30 S-dimers could be that when the 30 S subunits are in association with another ribosomal particle (30 S or 50 S), they assume a configuration different from that of the free 30 S and not compatible with a *stable* interaction with IF-3. Evidence has been presented that the 30 S particle undergoes a configurational change during association with 50 S subunits [18] and the importance of the configuration of the 30 S particles in the binding of IF-3 has already been pointed out [17]. The ability of IF-3 to induce a

configurational transition of the 30 S subunits will be described in a forthcoming paper [9].

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